

Met	Glu	Ser	Ile	Ile	Val	Gly	Ala	Gly	Thr	Phe	Gly	Leu	Ser	Thr	Ala	Leu	Gln	Leu	Ala	Arg	Asp	Gly	Tyr	Lys	Asn	Ile	Lys	Cys		
1	ATGGAGTGG	TAATTATAGT	TGGTGGCGGT	ACTTTGGGC	TTTCCACAGC	CTTACAGCTT	GCCAGAGATG	GATACAAGAA	CATAAAATGT																					
	Phe	Asp	Lys	Phe	Pro	Val	Pro	Ser	Glu	Ile	Ala	Ala	Gly	Asn	Asp	Ser	Lys	Ile	Phe	His	Tyr	Asp	Tyr	Val	Ala	Pro	Leu	Ala	Lys	
91	TTTGACAAGT	TTCCGGTTCC	ATCTGAGATA	GCTGCTGGAA	ACGACAGTAA	CAAGATTITT	CACTACGATT	ATGTTGCTCC	CCTGGCTAAA																					
	Pro	Asn	Ser	Lys	Glu	Arg	Leu	Ser	Leu	Glu	Ala	Leu	His	Leu	Trp	Lys	Thr	Asp	Pro	Val	Tyr	Lys	Pro	Tyr	Tyr	His	Pro	Val	Gly	Phe
181	CCCAATTCAA	AAGAACGGTT	GAGTCTCGAA	GCATTACACC	TTTGGGAAGC	AGATCCGGTG	TACAARCCGT	ACTATCATCC	GGTAGGATT																					
	Ile	Leu	Ala	Ala	Ser	Ser	Asp	Ala	Pro	Leu	Leu	His	Asp	Lys	Glu	Tyr	Tyr	Glu	Glu	Leu	Gln	Lys	Asn	Gly	Leu	Arg	Asn	Tyr	Arg	Tyr
271	ATCTGGCTG	CAAGTTCGGA	TGCTCCATT	CTGCATGATA	AGGAATACTA	TGAAGAGTTG	CAAAAAACG	GACTTCGCAA	TTATCGTTAT																					
	Ile	Ser	Thr	Pro	Glu	Glu	Phe	Arg	Glu	Tyr	Leu	Pro	Ile	Leu	Lys	Gly	Pro	Leu	Pro	Asn	Trp	Arg	Gly	Tyr	Val	Leu	Asp	Gly	Asp	Asn
361	ATTTCAACTC	CCGAGGAGTT	TCGTGAGTAT	TTGCCCATTT	TAAAGGGCCC	GTTACCCAAC	TGGAGAGGAT	ATGTTCTCGA	CGGAGATAAC																					
	Gly	Trp	Leu	His	Ala	Arg	Asp	Ser	Leu	Lys	Ser	Ala	Tyr	Glu	Cys	Lys	Arg	Leu	Gly	Val	Glu	Phe	Val	Phe	Gly	Asp	Asp	Gly	Glu	
451	GGATGTTGC	ATGCTCGAGA	CTCATTGAAA	AGTGCATACG	AAGAATGCAA	ACGATGCGGA	GTGGAATTG	TGTTGGAGA	CGATCGGGAA																					
	Ile	Val	Glu	Leu	Leu	Asn	Glu	Asn	Gly	Lys	Leu	Thr	Gly	Ile	Arg	Ala	Arg	Ser	Gly	Ala	Ile	Phe	Ser	Ala	Gln	Lys	Tyr	Val	Leu	Ser
541	ATTGTGGAAT	TACTTAACGA	AAATGGAAAG	TTGACGGGAA	TTAGGGCCAG	ATCTGTGCC	ATATTCTCGG	CACAAAATA	TGTTCTCAGC																					
	Ser	Gly	Ala	Asn	Ala	Val	Thr	Leu	Leu	Asn	Phe	Gln	Arg	Gln	Leu	Glu	Gly	Lys	Cys	Phe	Thr	Leu	Ala	His	Phe	Lys	Val	Thr	Asp	Glu
631	TCTGTGCAA	ATGCAGTAAC	GTGTGTAAT	TTCCAGAGAC	AGCTAGAAGG	TAAATGTTTC	ACTTTGGCAC	ATTTCAAAAT	GACGATGAA																					
	Glu	Ala	Lys	Ala	Phe	Lys	Ser	Leu	Pro	Val	Leu	Phe	Asn	Ala	Glu	Lys	Gly	Phe	Phe	Phe	Glu	Ala	Asp	Glu	Asn	Asn	Glu	Ile	Lys	Ile
721	GAAGCTAAAG	CATTAAAG	CTTGCCGGTC	CTTTCAATG	CCGAAAAGG	GTTTTTTTC	GAGGCTGATG	AAAATAACGA	AATCAAAATT																					
	Cys	Asn	Glu	Tyr	Pro	Gly	Phe	Thr	His	Thr	Asn	Glu	Ser	Gly	Glu	Ser	Ile	Pro	Leu	Tyr	Arg	Met	Glu	Ile	Pro	Leu	Glu	Ser	Ala	Leu
811	TGCAACGAGT	ACCCTGGATT	TACCCACACA	AATGAATCCG	GAGAGTCTAT	CCCACCTCTAC	CGGATGGAGA	TTCCACTCGA	GTACGCACTT																					
	Glu	Ile	Arg	Gln	Tyr	Leu	Lys	Glu	Thr	Met	Pro	Gln	Phe	Ala	Asp	Arg	Pro	Phe	Thr	Lys	Thr	Arg	Ile	Cys	Trp	Cys	Thr	Asp	Ser	Pro
901	GAATTAGAC	AATACTTGAA	AGAAACCATG	CCTCAGTTTG	CTGATAGACC	TTTCACCAAG	ACAAGAATTT	GTGGTGTAC	CGACTCTCCC																					
	Asp	Met	Gln	Leu	Ile	Leu	Cys	Thr	His	Pro	Glu	Tyr	Thr	Asn	Leu	Ile	Val	Ala	Ser	Gly	Asp	Ser	Gly	Asn	Ser	Phe	Lys	Ile	Met	Pro
991	GACATGCAAT	TGATCTTGTG	TACTCACCCA	GAATACACCA	ACCTTATTGT	AGCATCGGGT	GACTCTGGAA	ATTGTTTCAA	GATCATGCCA																					
	Ile	Ile	Gly	Lys	Tyr	Val	Ser	Lys	Val	Val	Thr	Lys	Gly	Asp	Lys	Gly	Leu	Asp	Pro	Glu	Asp	Lys	Glu	Cys	Trp	Lys	Trp	Arg	Pro	Glu
1081	ATCAITGGCA	AATATGTCAG	CAAGGTTGTT	ACCAAAGGTG	ATAAAGGATT	GGATCCGGAA	GATAAAGAAT	GCTGGAAATG	CGGTCCTGAG																					
	Thr	Trp	Asp	Lys	Arg	Gly	Gln	Val	Arg	Trp	Gly	Gly	Arg	Tyr	Arg	Val	Ala	Asp	Leu	Asn	Glu	Ile	Glu	Trp	Val	Ser	Val	Glu	Asn	
1171	ACTTGGGACA	ACGGGGGGCA	GGTCCGCTGG	GGTGCTCGAT	ACCGTGTTCG	GGATTGTAAC	GAATTTGAAG	AATGGGTTTC	TGTTGAAAAAT																					
	Pro	Thr	Pro	His	Lys	Leu	Glu	...																						
1261	CCCACACCA	ACAAACTAGA	ATAA																											

FIG. 1

mesiiivgagtfglstqlardgyknikfdkfpvpseiaagndsnkifhydyvaplakpnskerlsleahlwkt dpvykpyyhp  
 vgfilaassdapl lhdkeyyeelqknglrnyryistpeefreylpilkgplpnwrgyvldgdngwlhardslksayeckr lgvfv  
 fgddgeivellnengkl tgirarsgai fsaqkyvlssganavtllnfqrqlegkcftlahfkvtdeeakafkslpvlfnaekgfffe  
 adenneikicneypgfthtnesgesiplyrmeiplesaleirqylketmpqfadrpfktricwctdspdmqlilcthpeyntliva  
 sgdsngsfkimpigkyvskvvtkgdkgl dpedkecwkrpetwdkrqvrwggryrvadlneieewvsvenptphkle

FIG. 2

5' -atggagtcgataattatagttggcgccgtacttttgggctttccacagccctacagcttgccagagatggatacaagaacataaaatgtttgacaagttccggt  
 ccatctgagatagctgctggaaacgacagtaacaagattttcactacgattatgttgcctccctggctaaccctaattcaaaagaacggttgagctcgaagcattacac  
 ctgtggaagacagatccggtgtacaaaccgtactatcatccggtaggattatcctggctgcaagttccgatgctccattactgcatgataaggaatactatgaagattg  
 caaaaaacggacttcgcaattatcgttatattcaactcccaggagtttctgtagtatttgcctatitaaagggccgttaccctaactggagaggatatgttctgacg  
 gagataacggatgggtgcatgctcgagactcattgaaaagtgcatacgaagaatgcaaacgattgggagtggaattgtgtttggagacgatggggaaattgtcgaatt  
 acttaacgaaaatggaagttagcgggaattagggccagatctggcgcatattctcggcacaaaaatatgttctcagctctgggtgcaaatgcagtaacgtttgtaaat  
 ccagagacagctagaaggtaaatgtttcactttggcacatttcaaagtacggatgaagaagctaaagcattttaaagcttgcgggtcctttcaatgccgaaaaagggt  
 ttttttcgaggctgatgaaaataacgaaatcaaaattgcaacgagtagccctggatttaccacacaaatgaatccggagagtctatcccactctaccgatggagattc  
 cactcgagtcagcacttgaaattagacaatacttgaaagaaaccatgcctcagtttgcctgatagaccttccaccaagacaagaattgttgggtaccgactctcccgaca  
 tgcaattgatctgtgtactcaccagaatacaccaaccttattgtatgcacgggtgactctggaattcgttcaagatcatgccaatcattggcaaatatgtcagcaaggt  
 tgttaccaaagggtataaaggattggatccgggaagataaagaatgctggaatggcgctctgagacttgggacaagcgggggcagggtccgctggggtggtcgatac  
 cgtgttgcggattgaacgaaattgaagaatgggttctgttgaataccacaccacacaaactagaataa-3'

FIG. 3

FAO-F1 5'-GGXACXTGGGGXWSXWSXACXGCXYTXCA-3'  
FAO-R2 3'-TCYTCRTYXGGYTCVAWRAARAAXCC-5'  
in which \* S=C+G Y=C+T R=A+G X=A+C+G+T W=A+T V=A+C+G

FAO-F3 : 5'-ATTTCAAAGTGACGGATGAAGAAGCTAAAG-3'  
adaptor primer : 3'-CGCAGTTTTCCCAGTCACGAC-5'

FAO-F5 5'-GTGCATACGAAGAATGCAAACGATTGGGAGTGG-3'

FAO-R6 3'-CCATCCGTTATCTCCGTCGAGAACATATCCTC-5'

FAO-NcoI : 5'-ATCACCATGGAGTCGATAATTATAGTTGG-3'

FAO-XbaI : 3'-TTGATTCTAGACATGTATGTTGTAATCTTG-5'

FIG. 4

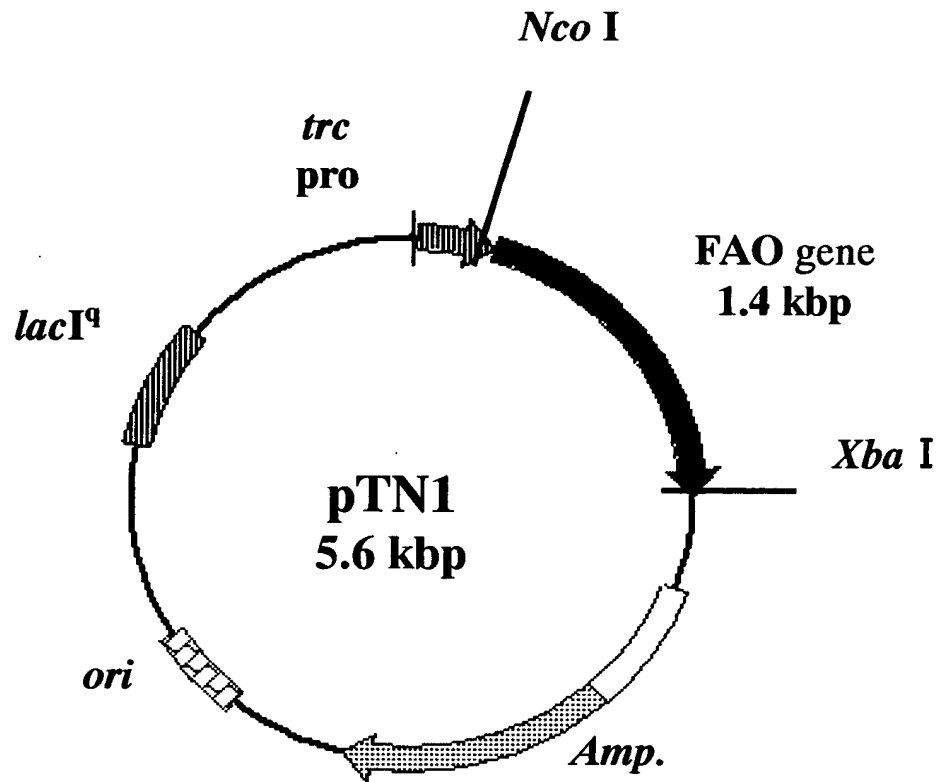


FIG. 5

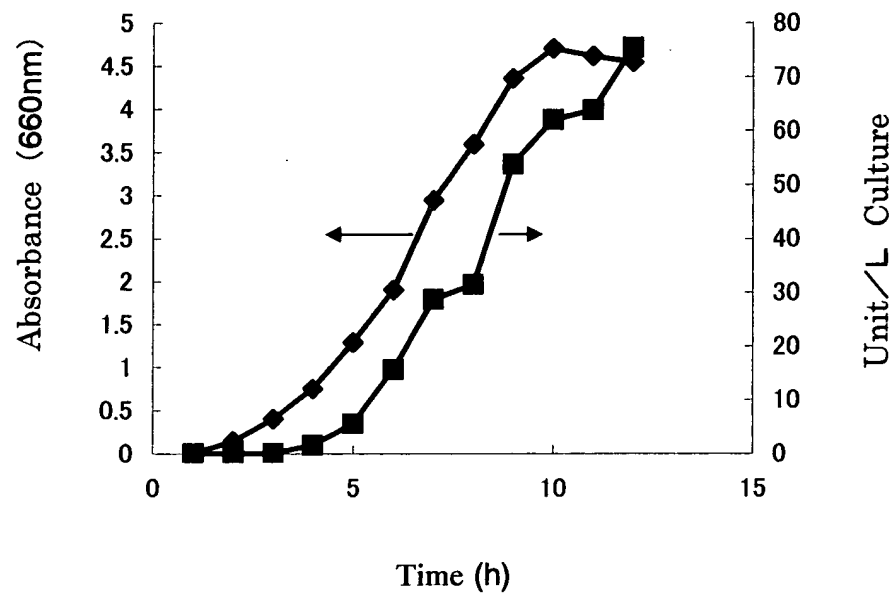
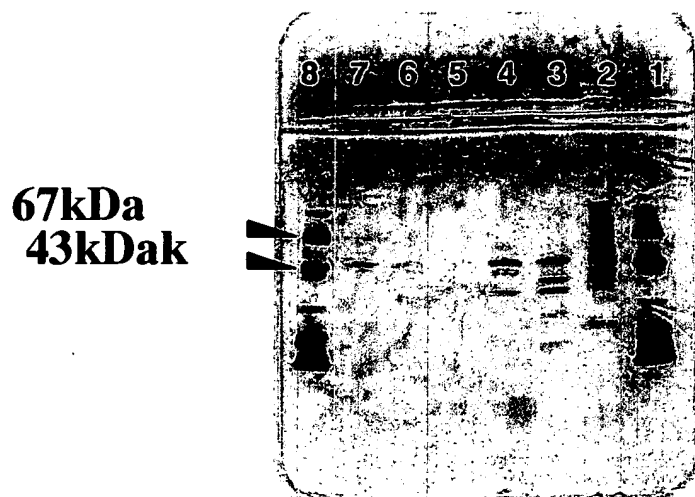


FIG. 6



- 1: LMW
- 2: Soluble
- 3: after DEAE 5PW
- 4: after RESOURCE Phe
- 5: after Bioasist Q No.23
- 6: after Bioasist Q No.24
- 7: after Bioasist Q No.25
- 8: LMW

FIG. 7

## Kinetic parameters

	Recombinant
<i>K<sub>m</sub></i> (mM)	5.9
<i>V<sub>max</sub></i> (U/mg)	7.1

## Substrate specificity

Substrate	Activity(%)	
	Recombinant	Wild type
fructosyl valine	100	100
fructosyl lysine	120	135
fructosyl glycine	4	9
fructosyl alanine	56	60
fructosyl leusine	14	31
fructosyl phenylalanine	104	103

FIG. 8